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(54) Title: ANTISENSE INHIBITION OF HUMAN ADHESION MOLECULES

(57) Abstract

The present invention provides a method for the treatment of human conditions and diseases that have an inflammatory component, including but not limited to acne, psoriasis, arthritis, transplanted organ rejection, wounds, burns, septic shock and inflammatory complications of shock. A process for selectively inhibiting the expression of the human ICAM-I, human E-selectin, or human VCAM-1 mRNA transcripts using at least one oligonucleotide which is complementary to at least a portion of the human ICAM-I, human E-selectin, or human VCAM-1 mRNAs is disclosed, as are oligonucleotides which are complementary to portions of the ICAM-I, human E-selectin, or human VCAM-1 mRNAs and compositions comprising the aforementioned oligonucleotides.

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ANTISENSE INHIBITION OF HUMAN ADHESION MOLECULES

FIELD OF THE INVENTION

This invention relates to the use of specific antisense oligonucleotides to inhibit the expression of proteins that mediate cell to cell association. Particularly, this invention relates to the inhibition of cellular adhesion molecules through the application of antisense oligonucleotides. More specifically, this invention relates to the use of certain sequence specific antisense oligonucleotides complementary to human mRNAs or pre-mRNAs coding for Intercellular Adhesion Molecule-1, ICAM-1, Endothelial Leukocyte Adhesion Molecule-1, E-selectin, and Vascular Cell Adhesion Molecule-1, VCAM-I. Most specifically, this invention relates to the use of antisense oligonucleotides target in human ICAM-1, E-selectin, or VCAM-1 for treating or preventing human diseases or conditions related to inflammation.

BACKGROUND OF THE INVENTION

Inflammation is characterized by the local accumulation of leukocytes, plasma proteins, and fluid usually at an extravascular site. Inflammatory processes are intrinsically destructive to the surrounding tissues and may, in certain circumstances such as allograft rejection or sepsis, be more harmful than beneficial. Thus, an appropriate strategy for evaluating conditions associated with an inflammatory component is down-regulation, but not total ablation, of the inflammatory response. Down-regulation of specific cell adhesion receptors and/or ligands to the receptors will prevent or lessen the inflammatory mediated damage to endothelial cells in the vasculature.

The migration of leukocytes into tissues is the central event in the immune or inflammation response. This migration to and

subsequent emigration into the tissue is responsible for the successful host response to injury and infection. The leukocytes are also potentially harmful and contribute to the pathology of many inflammatory disorders. The precise mechanism of this injury is not known, but the generation of free oxygen radicals and release of proteolytic enzymes have been implicated and may act together in leukocyte induced endothelial cell damage (Varani, J. et al. (1989), Am. J. Path. 135: 429-436). Evidence for the leukocyte adhesion to endothelial cells has been attributed to specific surface proteins.

The expression of many essential cell adhesion proteins and receptors is involved in the activation of the inflammatory and immune response, as during the development of inflammation. Adhesion molecules are activated by various cellular mediators, exogenous or endogenous to the host, and therefore, the logical approach is down-regulation of adhesion protein expression as opposed to treatments aimed at the multiple activators. Thus, the use of antisense oligonucleotides to specifically down-regulate adhesion protein expression offers obvious advantages to evaluating their role in inflammation.

A family of endothelial cell adhesion molecules known collectively as the immunoglobin superfamily are involved in the binding of leukocytes to activated endothelium. One member of this family is the intercellular cell adhesion molecule-1, also known as ICAM-1 (Springer, T.A., et al. (1987) A. Rev. Immun. 5: 223-252). ICAM-1 is a 90 kD cell-surface glycoprotein of endothelial cells that binds neutrophils and perhaps monocytes via the Lymphocyte Function Associated- 1, LFA-1, integrin expressed by white blood cells (Kishimoto, T.K. et al. (1989) Adv. Immunol. 46: 149-182). There are two pathways for the adhesion of leukocytes to endothelium: 1) an immediate adhesion that is not dependent upon the de novo synthesis of proteins, and 2) a delayed adhesion (1-2

hours) that is dependent upon the synthesis of proteins (Osborn, L. (1990) Cell 62: 3-6). The synthesis and surface presentation of ICAM-1 in endothelial cells suggests that ICAM-1 may be involved in the second, or delayed, component of leukocyte adhesion. Although ICAM-1 is present at low levels on both epithelium and endothelium under normal conditions, ICAM-1 gene or mRNA expression is markedly increased by a variety of inflammatory stimuli such as interferons, interleukins, i.e. interleukin-1, and tumor necrosis factor-alpha, TNF- α (Prober, J.S. and R.S. Cotran (1990) Transplant. 50: 537-544).

Stimulation of human umbilical vein endothelial cells (HUVECs) with either interleukin-1, interferon gamma, or tumor necrosis factor-alpha results in more than a 30-fold increase in the surface presentation of ICAM-1 (Osborn, L. et al. (1990) Cell 62: 3-6). Also, the stimulation of ICAM-1 synthesis by cytokines requires approximately a two hour delay and then a sustained increased presentation of the endothelial cell surface (Smith, C.W. et al (1988) J. Clin, Invest. 82: 1746-1756). This two hour delay in presentation of ICAM-1 correlates with the time course for adherence of neutrophils to endothelial cells in vivo (Munro, J.M. et al. (1991) Lab. Invest 64: 295-299). After 24 hours the expression of ICAM-1 and the ability of ICAM-1 to be recognized by the antibodies declines to pre-inflammation levels (Munro et al. supra). Thus, this study demonstrates that the early dermal accumulation of neutrophils may be associated with the endothelial cell expression of ICAM-1. These in vivo effects closely parallel the in vitro evidence concerning the induction of ICAM-1 and the role of ICAM-1 in neutrophil adhesion. The ability of antibodies to ICAM-1 to block the adhesion of neutrophils, eosinophils, and basophils induced in vitro in HUVECs by interleukin-1 suggests that ICAM-1 plays an important role during the inflammatory response

(Bochner, B.S. et al. (1991) J. Exp. Med. 173: 1553-1556 and Carlos, T. et al. (1991) Blood 77: 2266-2271).

The ability to target the pre- or mature-mRNA with antisense oligonucleotides with the express purpose of down-regulating the synthesis of ICAM-1 is immediately possible. The mRNA coding for ICAM-1 has been cloned and the nucleic acid sequence is available for selective targeting with antisense oligonucleotides (Staunton, D.E. et al., Cell 52-925-933; Wawryk, S.D. et al., Intl. Immunol 3: 83-93).

Evidence for the leukocyte adhesion to endothelial cells has been attributed to specific surface proteins. Another family of endothelial cell adhesion molecules known as selectins or LECCAM's is involved in the binding of leukocytes to activated endothelium. One member of this family is E-selectin. E-selectin is a 110 kD cell-surface glycoprotein of endothelial cells that binds neutrophils, and perhaps monocytes (Bevilacqua, M.P., et al., 1987, Proc. Natl. Acad. Sci. USA 84: 9238-9242). Of the two pathways for the adhesion of leukocytes to endothelium, immediate and delayed adhesion, the synthesis and surface presentation of E-selectin in endothelial cells suggests that E-selectin may be involved in the second, or delayed, component of leukocyte adhesion (Bevilacqua, J.S., et al., 1987, Proc. Natl. Acad. Sci. USA 84: 9238-9242 and Bevilacqua, J.S. et al, 1989, Science 243: 116-1165). Several mediators of an inflammatory response can increase the adhesion of leukocytes to endothelial cells through the biosynthesis and expression of E-selectin. Stimulation of HUVECs with either IL-1 or TNF- α results in more than a 100-fold increase in the surface presentation of E-selectin (Osborn, L. et al., 1990, Cell 62: 3-6). Also, the stimulation of E-selectin synthesis and its presentation on the surface of HUVECs has been shown to be mediated by endotoxin (Munro, J.M. et al., 1991, Lab. Invest. 64: 295-299). Injection of

endotoxin into the skin of baboons results in the strong, widespread endothelial binding of anti-E-selectin antibodies in the venules within 2 hours of injection. This two hour delay in presentation of E-selectin to the venules correlates with the time course for adherence of neutrophils. After 9 hours the expression of E-selectin and the ability of E-selectin to be recognized by the antibodies declines to pre-injection levels (Munro, J.M. et al. IBID). These results demonstrate that the early dermal accumulation of neutrophils after injection of endotoxin is associated with the endothelial cell expression of E-selectin. These in vivo effects closely parallel the in vitro evidence concerning the induction of E-selectin by endotoxin and the role of E-selectin in neutrophil adhesion. The ability of antibodies directed to E-selectin to block the adhesion of neutrophils, eosinophils, and basophils induced in vitro in HUVECs by interleukin-1 suggests that E-selectin plays an important role during the inflammatory response (Bochner, B.S. et al, 1991, J. Exp. Med. 173: 1553-1556 and Carlos, T. et al., 1991, Blood 77: 2266-2271).

E-selectin cDNA and genomic clones have been isolated and the nucleic acid sequence of the pre- and mature mRNA can be determined from these sequences (Goelz, S.E. et al., 1990, Cell 63: 1349-1355; Hession, C. et al., 1990, Proc. Natl. Acad. Sci. USA 87: 1673-1677; and Collins, T. et al., 1991, J. Biol. Chem. 266: 2466-2473).

There are many lines of evidence that indicate that inflammatory reactions are modulated by the interaction of circulating leukocytes with adhesion molecules on the endothelial cells of the luminal surface of blood vessels. These vascular adhesion molecules arrest circulating leukocytes and, thus, perform the first step in recruitment of these cells to sites of inflammation. Two cytokine inducible adhesion molecules, ICAM-1 and

E-selectin, found on the surface of the leukocytes have been characterized as important to the recruitment of circulating leukocytes to the sites of inflammation (Simmons, D., et al. (1988) Nature 331: 624-627; Staunton, D.E., et al. (1988) Cell 52: 925-933; Staunton, D.E., et al. (1989) Nature 339: 61-64). However, neither ICAM-1 or E-selectin appear to be involved in the recruitment or adhesion of lymphocytes, B- or T-cells, to activate endothelial cells. E-selectin is selective for PMNs, and perhaps, monocytes, but does not bind lymphocytes (Bevilacqua, M.P. (1987) Proc. Natl. Acad. Sci. USA 84: 9238-9242; Bevilacqua, M.P. (1989) Science 243: 1160-1165). ICAM-1 is the ligand for the leukocyte integrin receptor molecule, LFA-1, however, antibodies against LFA-1 do not block the adhesion of lymphocytic cells to activated endothelial cells (Haskard, D., et al. (1986) J. Immunol. 137: 2901-2906). These results suggest another pathway for lymphocyte adhesion to activated endothelium.

A prominent feature of endothelial cell activation by cytokines is the alternation of their surface adhesive properties due to the induction of adhesion molecules expression (Pober, J.S. and R.S. Cotran (1990) Physiol. Rev. 70: 427-451). The induction of these molecules leads to the hyperadhesive surface changes observed in vivo during various physiological conditions. VCAM-1 was identified in activated human umbilical endothelial cells by expression cloning (Osborne, L., et al. (1989) Cell 59: 1203-1211) and specific monoclonal antibodies (Rice, G.E. and M.P. Bevilacqua (1989) Science 246: 1301-1306). VCAM-1 is an inducible endothelial cell surface molecule which has been demonstrated to mediate intercellular adhesion via interaction with the integrin VLA-4, which is expressed on monocytes, lymphocytes, basophils, eosinophils, and certain tumor cells, but not neutrophils (Elices, M.J., et al. (1990) Cell 60: 577-584; Taichman, D.B., et al. (1991)

Cell Regul. 2: 347-356; Bochner, B.S., et al. (1991) J. Exp. med. 173: 1553-1556; Rice G.E., et al. (1990) J. Exp. Med. 171: 1369-1374). VCAM-1 expression is inducible on vascular endothelium in pathological conditions, however, it is constitutively expressed on some nonvascular cells (Rice, G.E., et al. (1990) J. Exp. Med. 171: 1369-1374; Rice, G.E., et al. (1991) AM. J. Pathol. 138: 385-393). In the inflammatory process, VCAM-1 is up-regulated at the level of translation on endothelial cells of the post-capillary venules (Briscoe, D.M., et al. (1991) Transplantation 51: 537-547). VCAM-1 is a transmembrane protein and a member of the immunoglobin gene superfamily. The VCAM-1 molecule comprises six immunoglobin-like domains which interact with the VLA-4 receptor on lymphocytes. Several lines of evidence are consistent with an important role for VCAM-1 in lymphocyte recruitment in the inflammatory process. 1) VCAM-1 expression is rapidly induced by IL-1 and TNF- α and this induction is sustained for up to 72 hours. This time course of VCAM-1 induction parallels the sustained mononuclear lymphocytic infiltration that occurs in delayed hypertension reactions (Dvorak, H.F., et al. (1980) Int. Rev. Exp. Pathol. 21: 195-199). 2) HUVECs express ICAM-1 and E-selectin upon exposure to cytokines and their expression occurs at sites of cytokine injection in vivo (Cotran, R.S. and Pobar, J.S. (1988) In Endothelial Cell Biology, N. Simionescu and M. Simionescu, eds. (New York: Plenum Press), pp. 335-347). HUVECs also express VCAM-1 upon exposure to cytokines (Osborne, L., et al. (1989) Cell 59: 1203-1211). 3) Frozen sections of human synovium exhibit the capacity to bind lymphocytes to inflamed vessels and not normal vessels, consistent with the presence of inducible VCAM-1 at these sites. Overall, these results suggest that VCAM-1 may be the central mediator of lymphocyte recruitment to the sites of inflammation in vivo. While the role of VCAM-1 in the adherence of resting T-cells

to interleukin-1 stimulated endothelial cells has been suggested, there is little evidence that VCAM-1 is involved in the binding of either activated T-cells or in the transendothelial migration of T-cells (Oppenheimer-Marks, N., et al. (1991) J. Immunol. 147: 2913-2921). Also, VCAM-1 does not appear to play a vital role in the adherence of activated T-cells to endothelial cells.

The data suggests that it is possible to inhibit the binding of resting T-cells to either unstimulated or activated endothelium by disruption of the recognition that occurs between VCAM-1 and VLA-4. The use of monoclonal antibodies against either VLA-4 and/or VCAM-1 can be useful in preventing the recognition of the receptor/ligand pair for each other. Another approach for interfering with the recognition process is via a down-regulation of VCAM-1 synthesis and, thereby, a reduction in the presentation of this molecule to the surface of activated endothelial cells. Antisense oligonucleotides provide an attractive approach for inhibiting the presentation of VCAM-1 molecules to the surface of the endothelial cells.

The interaction of the antisense oligonucleotide with its target mRNA is highly specific as hybridization is determined by the sequence of bases complementary to the antisense oligonucleotide, or by the Watson/Crick base pairing of the two strands of nucleic acid. Thus, there are multiple points of contact between the antisense oligonucleotide and the mRNA target, which increases the specificity for hybridization to the correct sequence. The specificity derived from the Watson/Crick base pairing is not evident in traditional drugs that inhibit the activity of proteins or mimic their action. The side effects resulting from the use of drug therapies occur through interactions at a few contact points between the drug and various proteins that possess similar binding sites or sites of interaction. Such adverse effects will be eliminated with antisense

drugs. Experimental calculations based on the number of base pairs in the human genome and the frequency of base utilization predict that there would be a single complement to a 13-mer antisense oligonucleotide in the entire human genome (Ts'o, P.O.P. et al, Biological Approaches to the Controlled Delivery of Drugs, Vol. 507, Ann. N.Y. Acad. Sci.).

While duplex stability is determined by many factors, of which length is of high importance, there are many oligonucleotides shorter than 15 nucleotides that may possess the capacity to form sufficiently stable heteroduplexes with mRNA. For example, using the nearest-neighbor thermodynamics approach of Breslauer et al (1986) Proc. Natl. Acad. Sci. 83: 3746-3750 and Freier, et al (1986) Proc. Natl. Acad. Sci. 83: 9373-9377) to analyze the first 122 nucleotides at the 5' end for mRNA for human VCAM-1 for stability, there are many that would form stable duplexes at 37°C . Analysis for stable 12-mers reveals that (potentially 111 oligonucleotides) there are 9 oligonucleotides with a Tm (that temperature at which 50% of the molecules form duplexes) greater than 50°C and 18 greater than 45°C. Analysis of 13-mers (potentially 110 oligonucleotides) reveals that there are 20 with predicted Tms greater than 50°C and 53 greater than 45°C. For an oligonucleotide that possesses a Tm of 54.5°C, at 32°C few oligonucleotides would remain single stranded and not form duplexes with target mRNA. There are no data in the literature which definitively addresses the optimum Tm or length of oligonucleotide for optimizing antisense activity. If a 13-mer is sufficient to define a unique mRNA (based upon theoretical calculations) then a 13-mer with sufficient mRNA heteroduplex stability might prove to be the most optimum antisense oligonucleotide. Theoretical analysis of two 13-mers (Tms 53.1 and 51.6°C) targeting the VCAM-1 predicts that the approximate Tms of these oligonucleotides decrease about 9.2°C per mismatch (Tm =Td -

(1.2)(% mismatch). For longer oligonucleotides this decrease per mismatch is less (i.e. for a 21-mer (with a Tm of 70°C) the decrease would be approximately 5.4°C). Based upon this information, a 21-mer might form a sufficiently stable heteroduplex with a non-target mRNA even with two mismatches (the Tm would decrease for this non-specific hybrid from 70°C to about 59°C). For a 13-mer (Tm 51.6°C) two mismatches would decrease the Tm to approximately 32°C. Thus, shorter oligonucleotides have the potential for increased specificity.

Evidence for down regulation of protein synthesis by antisense oligonucleotides has been well documented in vitro (for reviews see van der Krol, A.R. et al., BioTechniques 6: 958-976; Cohen, J.S., Antiviral. Res. 16: 121-133).

A major issue to be addressed in the use of antisense oligonucleotides for in vivo treatment is the stability of the molecule to the action of nucleases. Use of unmodified oligonucleotides containing phosphodiester linkages has not proven valuable in antisense therapy since these oligonucleotides are susceptible to exo- and endonucleases present in serum and cells. Thus, modification of the natural, or phosphodiester deoxyoligonucleotides (PO-ODN) have been developed that provide increased stability to degradation (Uhlmann, E. and A. Peyman, Chemical Reviews 90: 543-584). One of these modifications is the replacement of one of the non-bridging oxygen atoms in the phosphodiester linkage with sulfur to produce a phosphorothioate deoxyoligonucleotide (PS-ODN). The introduction of sulfur atoms in the PS-ODNs does not disrupt hybridization significantly. PS-ODN's retain the relative solubility of PO-ODNs in aqueous media and provide significantly enhanced nuclease stability to serum and cellular nucleases (Stein, C.A. et al., Nuc. Acids Res. 16: 3209-3221; Campbell, J.M., J. Biochem, Biophys. Methods 20:

259-267). Other modifications include methylphosphonates, phosphordithioate, sugar modifications, and heterocycle modifications (Goodchild, J., Bioconjugate Chem 1: 165-186). However, as novel chemistries become available there may be many new therapeutically useful chemical modifications of antisense oligonucleotides.

Recently, it has been reported that antisense PS-ODNs are capable of down-regulating the in vitro expression of one member of the immunoglobin superfamily adhesion proteins involved in cell-to-cell adhesion, ICAM-1 (Chiang, M-Y. et al., J. Biol. Chem 266-18162-18171, 1991). These data suggest that antisense oligonucleotides, particularly those stable to nucleases such as, for example, the phosphorothioate or 2'-O-methyl modified antisense oligonucleotides, are capable of inhibiting the expression of cell adhesion molecules. Thus, the use of antisense oligonucleotides in the down-regulation of ICAM-1 and other adhesion molecules should provide a basis for therapy and treatment of inflammatory disorders. Inhibition of the inflammatory response component may provide the necessary protection to prevent the deleterious consequences brought about by the body's own immune system.

SUMMARY OF THE INVENTION

Accordingly, an object of the present invention is the identification of antisense oligonucleotides that possess the capacity to inhibit the synthesis of human ICAM-1, E-selectin and VCAM-1.

An additional object of this invention is to provide an approach to evaluate the role these molecules play in the inflammatory process by inhibiting the expression of these proteins *in vitro* and *in vivo* using antisense oligonucleotides.

The foregoing objects of the present invention are

accomplished by providing antisense oligonucleotides that inhibit the expression of ICAM-1, E-selectin, and VCAM-1.

DESCRIPTION OF THE PREFERRED EMBODIMENT

A number of terms are known to have differing meanings when used in the literature. The following definitions are believed to be the ones most generally used and are consistent with the usage of the terms in the present specification.

The term "antisense oligonucleotides" is used to mean any natural or modified oligonucleotide or chemical entity that binds specifically to a pre-mRNA or mature mRNA which results in interference or inhibition with translation of the mature mRNA or prevents the synthesis of the polypeptide encoded by the mature mRNA.

"ICAM-1" as used herein describes the Intercellular Adhesion Molecule-1 involved in the adherence of leukocytes to endothelial cells.

"E-selectin" as used herein describes the Endothelial Leukocyte Adhesion Molecule-1 involved in the adherence of leukocytes to endothelial cells.

"VCAM-1" as used herein describes the Vascular Cell Adhesion Molecule - I involved in adherence of leukocytes (monocytes and neutrophils especially).

"mRNA" refers to mature, processed mRNA (messenger ribonucleic acid) or unprocessed, nuclear pre-mRNA transcribed from the gene(s) encoding for the synthesis of specific protein, such as ICAM-1, E-selectin, or VCAM-I. These sequences of ribonucleic acid are used to select the antisense oligonucleotide sequences which are complementary to discrete portions of the mRNA or pre-mRNA. The term "mRNA" is used herein to indicate either the mature of processed mRNA; or the unprocessed nuclear pre-mRNA.

As used herein, unless otherwise indicated, the term "oligonucleotide" includes both oligomer of ribonucleotides i.e., oligoribonucleotides, and oligomer of deoxyribonucleotides i.e., oligodeoxyribonucleotides, or oligodeoxynucleotides. Unless otherwise indicated, the term "oligonucleotide" also includes oligomers which may be large enough to be termed "polynucleotides".

The term "oligonucleotide" includes oligomer and polymers of biologically significant nucleotides, adenine, deoxyadenine, guanine, deoxyguanine, thymine, uracil, cytosine and deoxycytosine, as well as oligomers and polymers which contain other novel nucleotides and are hybridizable to the target mRNA transcript. These terms also include oligomers and polymers having one or more purine or pyrimidine moieties, sugar moieties, or internucleotide linkage(s) which has or have been chemically modified. This term may be used to include olgionucleotides that are comprised of modifications to the phosphodiester backbone including, but not limited to, phosphorothioate, methylphosphonate, or 2',5' linkages of normal phosphodiester or modified phosphodiester nature. Such modifications may be substantial and may encompass nonnucleotide chemistries including non-sugar, non-phosphate backbone, and chemical alterations to the bases to maintain the specific hybridization to the mRNA by base-pairing mechanisms, similar to or different from Watson-Crick base pairing. These terms further include those oligomers and polymers that are composed of nucleoside containing bases joined to the sugar moieties in the alpha configuration.

The term "downstream" is used herein to indicate the 5' to 3' direction in a nucleotide sequence. Similarly, the term "upstream" indicates the 3' to 5' direction.

The term "complementary" is used herein to indicate that the oligonucleotide is capable of hybridizing to and forming a stable

heteroduplex with its complementary sequence in the mRNA transcript with a Tm of at least 35°C under in vitro conditions of physiological ionic strength.

In accordance with the present invention, oligonucleotides having base sequences capable of hybridizing to the mRNA transcripts of the human ICAM-1, E-selectin, or VCAM-I adhesion receptors are provided. Hybridization of the oligonucleotide to the mRNA substantially blocks the translation of the mRNA transcript. Because ICAM-1, E-selectin, and VCAM-I are essential for the initial attachment or adhesion of leukocytes arising from stimulations that induce inflammation, down-regulation of the expression of one, both, or all three of these adhesion molecules may be mediated through antisense technologies.

The oligonucleotides of the present invention are constructed and purified by methods known in the art. The oligonucleotides may be prepared by solid phase or solution phase synthesis in an automated DNA synthesizer or by solution phase techniques.

Example 1:

Antisense oligonucleotides were synthesized using standard published techniques for the synthesis of phosphorothioate (PS) oligonucleotides. This oligonucleotide chemistry was employed to identify active sequence locations on the mRNA molecules for ICAM-1, E-selectin, or VCAM-1 and not intended to be the sole modification employable under the scope of this invention. Preferably, synthesis of antisense oligonucleotides is performed using a solid support and a commercially available DNA synthesizer. Antisense oligonucleotides were synthesized using standard phosphoramidite chemistry. For phosphodiester linkages the oxidation may be mediated via iodine, while for the synthesis of these

phosphorothioates, the oxidation was mediated with a 0.2 M solution of 3H-1,2-benzodithiole-3-one,1-dioxide in acetonitrile (Iyer, R.P., et al. (1990) J. Amer. Chem. Soc. 112: 1253-1254) for the step-wise thioation of the phosphate linkages. The thioation step is increased to 68 sec and is followed by a capping step. Following synthesis and cleavage from the control pore glass support, the trityl-on oligonucleotide is purified using HPLC. HPLC methodologies consisted of chromatography using a PRP-1 column and gradient of acetonitrile in 50 mM triethylammonium acetate, pH 7.0 (4-32% in 30min, flow rate of 1.5 ml/min). Appropriate fractions were pooled, evaporated, and treated with 5% acetic acid for 15 min at ambient temperature. The oligonucleotide solution was extracted with an equal volume of ethyl acetate, neutralized with ammonium hydroxide, frozen and lyophilized. Solution based chemistries are also useful for synthesis of antisense oligonucleotides and are useful of scaled-up synthesis of oligonucleotides.

In a less preferred method, the oligonucleotide may be prepared through the use of reverse transcriptase, PCR synthesis or via other genetic engineering techniques. The method of preference is an automated DNA synthesis on a solid phase support, however, any method of synthesizing oligonucleotides may be used. The specific oligonucleotide sequences are such that they are complementary to either the ICAM-1, E-selectin, or VCAM-I mRNAs or genes. In particular, the oligonucleotide sequences were complementary to the transcripts including the translation initiation codons, and sequences 5' and/or 3' to the translation initiation sites,. of the 5' cap region of the mRNAs and sequences 3' to the cap sites. Other oligonucleotide sequences. were also made complementary to the 3' untranslated region of the genes. Although not tested, it is anticipated by this invention that modifications to antisense oligonucleotides could include cross-linking DNA or intercalating

DNA moieties. Furthermore, the invention contemplates that other oligonucleotides or combination of oligonucleotides capable of specifically and substantially inhibiting the expression of ICAM-1, E-selectin, or VCAM-I can be used.

The oligonucleotides of this invention comprise predetermined sequences of DNA ranging in size from about 10 bases up to about 30 bases, which is sufficient to define a unique sequence the human target mRNA transcripts. Alternatively, two or more oligonucleotides of 7-15 bases may be joined together by non-nucleotide linkages. For such oligonucleotides less than 14 bases may be used, however the degree of sequence uniqueness decreases rapidly with decreasing length and thereby greatly reduce the specificity of the oligonucleotide for the target mRNA transcript. On the other hand, oligonucleotide sequences greater than about 30 bases may be subject to decreased cellular uptake and have an increased likelihood of containing short stretches of nucleotide sequence capable of forming quasi-stable hybrids with non-target mRNA sequences, other than one of the targeted mRNA transcripts. It is preferable that the oligonucleotides comprise about 14 to 22 bases and most preferably, a 21-mer oligonucleotide is used.

Example 2:

HUVEC were cultured to confluence in 48 well plates of 8 well chamber slides using media described by The American Types Culture Collection (ATCC, Rockville, MD). After washing the cells four times with 200 µl Opti-MEM (Life Technologies, Frederick, MD), HUVEC in duplicate or triplicate wells were treated for 2 hours at 37°C with Lipofectin (Life Technologies, Frederick, MD) at 10 µg/ml in Opti-MEM, immediately followed by addition of concentrated antisense phosphorothioate oligodeoxynucleotides (PS-ODN) in

phosphate buffered saline with Ca^{2+} (PBS). Stimulation of the cells for 4-6 hours at 37°C with 0.1 - 1.0 μ g/ml lipopolysaccharide (LPS), 20 - 80 units/ml (SA: >2 x 10^7 units/mg TNF-a, or 5 - 10 units/ml (SA: > 1 x 10^7 units/mg) IL-1b was carried out before, after, or at the same time as PS-ODN treatment depending upon the design of the experiments. The control samples of 100% expression were treated with stimulant but without PS-ODN, while those of 0% expression were treated with neither stimulant or PS-ODN.

Example 3:

Radioimmunoassay (RIA) was carried out as previously reported (Lee, C.H., Y.A. Reid, J.S. Jong, and K.H. Kang, 1995, Shock 3:96-101). This assay has an advantage over assays that measure the activity of the protein since it is a direct quantitation of the presence of the target protein. Thus, reductions in the amount of ICAM-1 , E-selectin, or VCAM-1 suggests that translation of the targeted protein's mRNA has been inhibited. Since the mechanism of antisense action is to interfere with mRNA translation, this is a better indicator of antisense activity.

After treatment and incubation, cells were chilled on ice and the medium was removed followed by addition to each well except the 0% control, which was added with 100 μ l of 2% sheep serum, with 100 μ l of murine anti-human ICAM-1, ELAM-1, or VCAM-1 antibody (R&D Systems, Minneapolis, MN) at 4 mg/ml in 2% sheep serum in PBS. The plate was incubated on ice for 1 hour and washed three (3) times with 200 μ l of PBS. Wells were treated with 100 ml of sheep anti-mouse IgG labeled with ^{125}I (DuPont, Boston, MA) in 2% sheep serum in PBS and then incubated on ice for an additional 1 hour. After washing for three (3) times with 200 μ l PBS, the cells in each well were disrupted with 500 ml of 0.1 N NaOH. The radioactivity was determined with 300 μ l of cell lysate by a gamma counter. The remaining lysate was used for protein determinations,

an index of the total number of cells in the sample. It was found that the protein content in the samples was not significantly altered throughout the cell treatment and the RIA procedure. This is suggestive of limited or no toxicity associated with PS-ODN dosing under our experimental procedures. After averaging the radioactivity of the duplicate or triplicate samples and subtracting the background, (i.e., the 0% control), the data of each treatment were normalized to the 100% control.

Example 4:

Antisense oligonucleotide treated HUVEC were incubated at 37°C for 1 hour with human monocytes (1 x 10^6 cells/well) or U937 cells (3 x 10^5 cells/well) previously labeled with [^{3}H]-thymidine or [^{51}Cr]. The wells were then washed four times with 200 µ l/well ice cold PBS. The cells in each well were disrupted with 250 µ l of 0.1 N NaOH followed by neutralization with 250 µ l of 0.1 N HCl. The lysates were then counted directly for gamma emission (51Cr) or mixed with 10 ml of scintillation cocktail for beta counting (^{3}H). After correction for background, the data were normalized to controls (100%); i.e., the samples treated with LPS, TNF-a, or IL1b, but without oligonucleotides.

For analysis of adhesion, cells grown on 8 well chamber slides were fixed with 2% glutaraldehyde, 1% paraformaldehyde in sodium cacodylate buffer (pH 7.2) for 1 hour. After washing 4 times, the samples were kept in the same buffer overnight at 4°C. The cell well divider was removed and the slides rinsed twice with de-ionized water and mounted with 10% glycerol and a coverslip. Coverslips were sealed with fingernail polish and then examined and photographed under using a light microscope.

Example 5:

These experiments utilized both Balb/c and C57BL/6 mice.

Both types of mice exhibited a reproducible inflammatory response following an i.p. challenge with 2 ml of 1.5% thioglycollate (TG). For experiments using antibodies specific for the various CAM molecules, mice were treated by injection into the tail vein 30 minutes prior to TG challenge with 200 μ l of PBS containing 50 μ g of antibody. For experiments using antisense oligonucleotides, 100 μ g oligonucleotide was administered in 200 μ l PBS through the tail vein 3-4 hours prior to TG challenge. This dose is approximately 5 mg/kg. Antisense treatment did not involve the use of any liposomal formulations or other delivery vehicles, such as Lipofectin (Life Technologies, Inc.). For mice treated with both antibodies and antisense oligonucleotides, compounds were administered as for single treatment mice. Four hours after TG administration, mice were sacrificed by cervical dislocation and the peritoneum was lavaged twice with 3 ml of PBS containing BSA, heparin, and EDTA. The lavages were combined and white blood cells were counted in an automated cell counter. Aliquots were centrifuged in a Cytospin, stained with H&E, and differentials obtained by microscopic examination. The percentages of PMN, lymphocytes, and macrophages were multiplied by total white blood cell counts to calculate the number of individual cell types.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 illustrates inhibition of monocytes (A, B, C, and D) adhesion to TNFa-stimulated HUVEC by .1 μ M PS-ODNs GM1508 (C), GM1520 (D), GM 1508 (G), and GM 1534 (H). The 100% controls (A and E) were treated only with TNFa, and the 0% controls (B and F) were treated with neither PS-ODN nor TNFa. Compared with the controls, remarkable reduction of cells adhering to HUVEC was shown in samples C and D for monocytes, G and H for U937.

DESCRIPTION OF FIGURE AND TABLES:

Many PS-ODN were tested for their ability to inhibit the expression of ICAM-1 in HUVEC. Of this group of oligonucleotides, ten (10) different sequences exhibited significant activity (greater than approximately 40% inhibition) at 0.1 μ M oligonucleotide concentration. Many of the tested sequences failed to convincingly produce a reduction in ICAM-1 induction. Based upon the assay above, these oligonucleotides exhibited significant activity in HUVEC for reducing the expression of ICAM-1. Regardless of the inducer chosen (LPS, TNF-a, or IL1b), antisense effects were consistent for active oligonucleotides. Antisense oligonucleotides that exhibited the capacity to reduce expression of ICAM-1 when cells were stimulated with one inducer, also possessed the capacity to inhibit expression when the other inducers were used. Thus, even though different pathways were used to stimulate ICAM-1 expression, the result, ICAM-1 expression was inhibited by active sequences. This is suggestive of a true antisense mechanism. Also, generally non-antisense effects of PS-ODN are observed at concentrations greater than 1.0 μ M. In order to identify an active sequence, we chose to use data derived from a single dosing of 0.1 μ M concentration. Non-antisense effects are generally observed two orders of magnitude (about 10 μ M) above the 0.1 μ M criteria used in our selection of active sequences. In Table 1 the percentage expressions by antisense oligonucleotides targeting ICAM-1 are listed for the above oligonucleotides when administered to HUVEC at 0.1 μ M concentrations.

From the data shown Table 1 sequences identified as being most potent at inhibiting ICAM-1 expression were Sequence ID No. 11, Sequence ID No. 2, and Sequence ID No. 7.

Table 1

Sequence ID No.	%Expression
	148 ± 11
	217 ± 08
	366 ± 07
	435 ± 12
	568 ± 06
	654 ± 12
	720 ± 16
	836 ± 08
	939 ± 05
	1033 ± 06
	11 7 ± 05
	1233 ± 07
	1361 ± 12
	1458 ± 16

Many PS-ODN were tested for their ability to inhibit the expression of E-selectin in HUVEC. Of this group of oligonucleotides, twelve (12) different sequences exhibited significant activity (about 40% inhibition) in the 0.1 μ M range. These are listed in Table 2. Based upon the assay above, these oligonucleotides exhibited significant activity in HUVEC for reducing the expression of E-selectin. Antisense oligonucleotides that exhibited the capacity to reduce expression of E-selectin when cells were stimulated with one inducer, also possessed the capacity to inhibit expression when the other inducers were used. Thus, even though different pathways were used to stimulate E-selectin expression, E-selectin expression, was inhibited by active

sequences. This is suggestive of a true antisense mechanism. Also, generally non-antisense effects of PS-ODN are observed at concentrations much greater than 1.0 μ M. In order to identify an active sequence, we chose to use data derived from a single dosing of 0.1 μ M concentration. In Table 2 the percentage expression for E-selectin is listed for the above oligonucleotides when administered to HUVEC at 0.1 μ M concentrations.

Table 2

Sequence ID #	%Expression
15	38 ± 11
16	10 ± 7
17	32 ± 14
18	51 ± 15
19	50 ± 15
20	8 ± 05
21	52 ± 05
22	50 ± 14
23	62 ± 14
24	51 ± 19
25	61 ± 16
26	63 ± 20

Antisense oligonucleotides have activity in reducing the expression of E-selectin when HUVEC are stimulated with various inducers of inflammation. Some oligonucleotides are more active than others. In the sequences identified as being active against E-selectin expression the most potent two were Sequence ID No. 16 and Sequence ID No. 20.

Many PS-ODN were tested for their ability to inhibit the expression of VCAM-1 in HUVEC. Within this group of oligonucleotides, twenty (20) different sequences exhibited significant activity (about 40% inhibition) in the 0.1 μ M range. These are listed in Table 3. Based upon the assay above, these oligonucleotides exhibited significant activity in HUVEC for reducing the expression of TNF-a induced VCAM-1. Antisense oligonucleotides that exhibited the capacity to reduce expression of VCAM-1 when cells were stimulated with one inducer, also possessed the capacity to inhibit expression when the other inducers were used. Thus, even though different pathways were used to stimulate VCAM-1 expression, the result, VCAM-1 expression, was inhibited by active sequences. This is suggestive of a true antisense mechanism. Also, generally non-antisense effects of PS-ODN are observed at concentrations much greater than 1.0 μ M. In order to identify an active sequence, we chose to use data derived from a dosing of 0.1 μ M concentration. In Table 3 the percentage expression for VCAM-1 is listed for the above oligonucleotides when administered to HUVEC at 0.1 μ M concentrations. Some oligonucleotides are more active than others. Of the above sequences identified as being active against VCAM-1 expression the most potent three were Sequence ID No. 28, Sequence ID No. 30 and Sequence ID No. 31.

Table 3

Sequence ID No.	%Expression
27	30 ± 13
28	9 ± 08
29	33 ± 05
30	5 ± 06
31	7 ± 06
32	12 ± 11
33	13 ± 02
34	25 ± 12
35	49 ± 09
36	20 ± 13
37	26 ± 19
38	11 ± 10
39	16 ± 09
40	32 ± 11
41	35 ± 13
42	39 ± 15
43	37 ± 20
44	42 ± 10
45	10 ± 07
46	45 ± 10

Pre-treatment of HUVEC with PS-ODN's led to a reduction of in the adherence of monocytes and U937 cells. Compared to 100% and 0% controls, monocytes and U937 adherence to the antisense (0.1 μ M oligonucleotide) treated HUVEC cells were significantly decreased (Figure 1). Using [^{51}Cr]-labeled monocytes and [^{3}H]-labeled U937 cells, antisense oligonucleotide -dependent cell adhesion was observed (Figure 2). In these experiments, oligonucleotides

Sequence ID No. 11 (ICAM-1), Sequence ID No. 20 (E-selectin), and Sequence ID No. 28 (VCAM-1), or Sequence ID No. 2 (ICAM-1) inhibited the adherence of monocytes or U937 cells to HUVEC. The dose response measuring cell adherence is parallel to the expression patterns of the various adhesion molecules following antisense treatment.

Prior to challenge with thioglycollate, lavage fluids only contained 90,000 PMN (in addition to 2.5 $x10^6$ lymphocytes and 1.2 x 10^6 macrophages). After TG challenge, the numbers of PMN rise over four hours to a peak of 9-11 x 10^6, and then slowly recede over the next 48-72 hours. The numbers of lymphocytes and macrophages increase to a much lesser extent. The infiltration of PMN responds to TG in a dose dependent fashion reaching a maximal response at 1.5 ml of 2% TG. Interperitoneal administration of antibody or antisense oligonucleotides was not found to be effective, so i.v. administration through the tail vein was utilized.

Treatment with antibodies directed against ICAM-1, CD11b, and CD18 all partially blocked PMN infiltration in this model system (Table 4). Treatment with antibodies directed at P-selectin and L-selectin were also partially effective. Treatment with antibody directed against E-selectin was marginally effective at best. Blockade of VCAM-1 had no effect (data not shown). In combination, blockade of ICAM-1 and CD18 or ICAM-1 and CD11b almost completely abolished PMN infiltration. Combination blockades of ICAM-1 and P-selectin and P-selectin or ICAM-1 and L-selectin were also very effective.

Since we did not have an appropriate cell-based model system to identify the most active antisense oligonucleotide targeting murine ICAM-1, a number of oligonucleotide sequences were synthesized targeting various regions of the mRNA. These sequences may not be the best selection for eliciting an antisense response. However, using control sequences containing scrambled or base-pair

mismatches sequences, the oligonucleotide-induce effect is much less (DPC5205, DPC 5206, and DPC5094, Table 5).

Treatment with antisense oligonucleotide DPC5028, a 21-mer directed at the AUG initiation codon of murine ICAM-1, was successful at inhibiting the adherence of PMN following TG challenge (Table 5). The effect of DPC5028 was dependent upon the time of administration. Oligonucleotide DPC5028 was effective when administered between 24 hours before and up to the time of TG challenge. At 48 hours prior to challenge and 2 hours post challenge, DPC5028 was without effect. However, due to varying sensitivities of the experimental subjects, there was intra-experimental variability. During these experiments, it was noticed that there was an age dependent incidence of skin inflammation in C57BL/6 mice that was later identified by Jackson Laboratories. Due to this complication, data generated from these animals was suspect. Also, the Balb/c mice exhibited an age dependent anergy, in that as these mice age there is a reduction in their response to TG challenge. Over a 30 day period the number of PMN was found to decrease by up to 25% following TG challenge. During this time period, there was an attenuation of the anti-ICAM-1 blockade evidenced by anti-ICAM-1 antibody or DPC5028 pre-treatment. In experiments conducted on younger Balb/c mice there was a significant (compared to TG treated mice) reduction in PMN adherence with DPC5028 treatment (Table 5). Combination treatment with anti-ICAM-1 and anti-p-selectin antibodies produced an additive reduction in PMN adherence (About 75%) compared to either antibody administered alone. When DPC5028 was administered concomitantly with antibody directed against P-selectin, there was a similar additive effect (about 60%).

This cumulative data presented above, suggests that antisense oligonucleotides capable of interfering with the

presentation of cellular adhesion molecules could have therapeutic benefit for humans by reducing the migration and adherence of cells involved in the inflammatory response. That antisense oligonucleotides targeting these cellular adhesion mRNAs are capable of working in humans similar to those used in the above in vivo demonstration in the murine model of inflammation, is evidenced by the reduction of adherence through antisense treatment of HUVEC in Figure 1.

Several reports in the literature suggest that phosphorothioate oligonucleotides can elicit sequence-specific effects through non-antisense mechanisms when the sequence contains either a CpG motif (for example see Kreig et al., 1995, Nature 374: 546) . The CpG motif can cause immune activation, particularly activation of B-cells and natural killer cells. DPC5028 does not contain any CpG motif so this sequence should not be inducing activation of the immune response. However, when two different sequences containing one (DPC5207) or three (DPC5083) CpG motifs were tested for their effects on PMN adhesion in this model system, there was a significant reduction in PMN adhesion (Table 5). Since DPC5028 and the other control oligos do not contain this motif, the activity of DPC5028 may be a result of antisense-mediated reductions in ICAM-1 expression.

Table 4
Antibody blockade of TG-induced PMN infiltration
grouped by experiment

Treatment	Blockade (% of ctrl)	Significance p<0.05
I		
ICAM-1	38	*
CD11B	50	*
E-selectin	86	
VCAM-1	100	
II		
P-selectin	65	*
ICAM-1	34	*
P-selectin + ICAM-1	13	*
III		
L-selectin	62	*
ICAM-1	49	*
L-selectin + ICAM-1	16	*
IV		
E-selectin	79	*
ICAM-1	49	*
E-selectin + ICAM-1	47	*
V		
ICAM-1	51	*
CD11b	40	*
CD18	12	*
CD11b + ICAM-1	9	*
CD18 + ICAM-1	4	*

TABLE 5

DPC No. Activity	Sequence Characteristics	(% of ctrl)
5028	Antisense AUG Region	68
5205	Scrambled 5028	90
5206	Scrambled 5028	86
5084	Scrambled 2 base mismatch of 5028	83
5207	Scrambled 5028 with 1 CpG	65
5083	Scrambled 5028 with 3 CpG	61

SEQUENCE LISTING

(1) GENERAL INFORMATION
(i) APPLICANT: Hoke, G.D., et al.
(ii) TITLE OF INVENTION: Antisense inhibition of human adhesion molecules
(iii) NUMBER OF SEQUENCES: 46
(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Max Oppenheimer
(B) STREET: P. O. Box 50
(C) CITY: Stevenson
(D) STATE: MD
(E) COUNTRY: US
(F) ZIP: 21153
(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.4 Mb STORAGE
(B) COMPUTER: MACINTOSH IICi
(C) OPERATING SYSTEM: MACINTOSH SYSTEM 7.0.1
(D) SOFTWARE: MICROSOFT WORD 5.1a

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER;
(B) FILING DATE:
(C) CLASSIFICATION:
(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Max Stul Oppenheimer
(B) REGISTRATION NUMBER: 33,203
(C) REFERENCE/DOCKET NUMBER: DYADPCT9602
(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 410-706-1793
(B) TELEFAX: 410-706-0407

(2) INFORMATION FOR SEQ. ID NO: 1
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 1
(xi) GCTTTCCCGG AAACCCTCGCG C

(3) INFORMATION FOR SEQ. ID NO: 2
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 2
(xi) CTCTGAGTAG CAGAGGAGCT C

(4) INFORMATION FOR SEQ. ID NO: 3
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 3
(xi) CCCCGACTCA CCTGGGAACA G

(5) INFORMATION FOR SEQ. ID NO: 4
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 4
(xi) GCTACACATG TCTATGGAGG G

(6) INFORMATION FOR SEQ. ID NO: 5
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 5
(xi) GGCAGAAATG TATGTGGGTG G

(7) INFORMATION FOR SEQ. ID NO: 6
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 6
(xi) ACACATACAC ACACACACAC A

(8) INFORMATION FOR SEQ. ID NO: 7
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 7
(xi) AGCCAGAGCG AGGCTGAGGT T

(9) INFORMATION FOR SEQ. ID NO: 8
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 8
(xi) GGTTGCAACT CTGAG

(10) INFORMATION FOR SEQ. ID NO: 9
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 17
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 9
(xi) CTATGGAGGG CCACTTC

(11) INFORMATION FOR SEQ. ID NO: 10
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 10
(xi) GGGCCACTTC TTCTGTAA

(12) INFORMATION FOR SEQ. ID NO: 11
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 11
(xi) CCCCCACCAC TTCCCCTCTC A

(13) INFORMATION FOR SEQ. ID NO: 12
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 22
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 12
(xi) TCTGAGTAGC AGAGGAGCTC AG

(14) INFORMATION FOR SEQ. ID NO: 13
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 13
(xi) GAGTAGCAGA GGAGC

(15) INFORMATION FOR SEQ. ID NO: 14
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 14
(xi) GATGCTACAC ATGTC

(16) INFORMATION FOR SEQ. ID NO: 15
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 15
(xi) GTTTAAGGCA GCATCCTAAG A

(17) INFORMATION FOR SEQ. ID NO: 16
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 16
(xi) TCACCCAAAG GTTTAGGCTT G

(18) INFORMATION FOR SEQ. ID NO: 17
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 33
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 17
(xi) GCAATCATGA CTTCAAGAGT T

(19) INFORMATION FOR SEQ. ID NO: 18
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 18
(xi) GTTCACACCT GAAAAAGAAA G

(20) INFORMATION FOR SEQ. ID NO: 19
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 19
(xi) GCATGTCACA GCTGTAACAA A

(21) INFORMATION FOR SEQ. ID NO: 20
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 20
(xi) TGAAGTCAGC CAAGAACAGC T

(22) INFORMATION FOR SEQ. ID NO: 21
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 21
(xi) CGTTCTGCAC TTACCGTTTT G

(23) INFORMATION FOR SEQ. ID NO: 22
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 22
(xi) CAGCCAAGAA CAGCT

(24) INFORMATION FOR SEQ. ID NO: 23
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 17
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 23
(xi) CAGCCAAGAA CAGCTGG

(25) INFORMATION FOR SEQ. ID NO: 24
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 17
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 24
(xi) GATGTGAAGT CAGCCAA

(26) INFORMATION FOR SEQ. ID NO: 25
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 25
(xi) CCCAAAGGTT TAGGCTTG

(27) INFORMATION FOR SEQ. ID NO: 26
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 26
(xi) GAGTTCTTTT CACCC

(28) INFORMATION FOR SEQ. ID NO: 27
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 27
(xi) GCCTGGGAGG GTATTCAGCT C

(29) INFORMATION FOR SEQ. ID NO: 28
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 28
(xi) ACCCCTTATT TGTGTCCCAC C

(30) INFORMATION FOR SEQ. ID NO: 29
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 29
(xi) CCCAGGCATT TTAAGTTGCT G

(31) INFORMATION FOR SEQ. ID NO: 30
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 30
(xi) CACGAGGCCA CCACTCATCT C

(32) INFORMATION FOR SEQ. ID NO: 31
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 31
(xi) CTTTGACTTC TTGCTCACAG C

(33) INFORMATION FOR SEQ. ID NO: 32
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 32
(xi) AACTCCTCCA GTTCTCTCAT C

(34) INFORMATION FOR SEQ. ID NO: 33
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 33
(xi) TCCTGAAGCC AGTGAGGCCC G

(35) INFORMATION FOR SEQ. ID NO: 34
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 34
(xi) ACCTGTGTGT GCCTGGGAGG G

(36) INFORMATION FOR SEQ. ID NO: 35
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 35
(xi) CGTGAGGAGA AAATAGTGGT T

(37) INFORMATION FOR SEQ. ID NO: 36
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 36
(xi) CGACCATCTT CCCAGGCATT T

(38) INFORMATION FOR SEQ. ID NO: 37
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 37
(xi) CCACCACTCA TCTCG

(39) INFORMATION FOR SEQ. ID NO: 38
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 18
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 38
(xi) CCCATTCACG AGGCCACC

(40) INFORMATION FOR SEQ. ID NO: 39
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 14
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 39
(xi) CGAGGCCACC ACTC

(41) INFORMATION FOR SEQ. ID NO: 40
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 14
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 40
(xi) CCTCCAGTTC TCTC

(42) INFORMATION FOR SEQ. ID NO: 41
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 14
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 41
(xi) GCCAGTGAGG CCCG

(43) INFORMATION FOR SEQ. ID NO: 42
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 42
(xi) CCTGGGAGGG TATTC

(44) INFORMATION FOR SEQ. ID NO: 43
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 16
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 43
(xi) CCCTTATTTG TGTCCC

(45) INFORMATION FOR SEQ. ID NO: 44
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 44
(xi) GGTTCCAAAA CCCTT

(46) INFORMATION FOR SEQ. ID NO: 45
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 45
(xi) TTTGTGTCCC ACCTG

(47) INFORMATION FOR SEQ. ID NO: 46
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 15
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(E) ANTI-SENSE: Yes
(iv) SEQUENCE DESCRIPTION: SEQ. ID NO: 46
(xi) CATCTTCCCA GGCAT

WHAT IS CLAIMED:

1. An oligonucleotide having a nucleotide sequence at least substantially complementary to at least a portion of the pre-mRNA or mature mRNA transcript of human ICAM-I, human E-selectin, or human VCAM-1, said oligonucleotides being hybridizable to said mRNA transcripts.

2. The oligonucleotide of Claim 1 which comprises a deoxynucleotide sequence at least substantially complementary to a portion of the human ICAM-I, E-selectin, or VCAM-1, mRNA transcripts immediately downstream from the first nucleotide of the mRNAs, known as the capsite.

3. The oligonucleotide of Claim 1 which comprises a deoxynucleotide sequence at least substantially complementary to a portion of the human ICAM-I, E-selectin, or VCAM-1 mRNA transcripts immediately downstream from the translation initiation codons of said transcripts.

4. The oligonucleotide of Claim 1 which comprises a deoxynucleotide sequence at least substantially complementary to a portion of the human ICAM-I, E-selectin, or VCAM-1 mRNA transcripts which comprises at least a portion of the 5'-untranslated region of said transcripts.

5. The oligonucleotide of Claim 1 which comprises a deoxynucleotide sequence at least substantially complementary to a portion of the human ICAM-I, E-selectin, or VCAM-1 mRNA transcripts which comprises at least a portion of the 3'-untranslated region of

said transcripts.

6. The oligonucleotide of Claim 1 which comprises at least 15 bases.

7. An oligonucleotide according to Claim 1 which comprises from a 15-mer to a 25-mer.

8. An oligonucleotide according to Claim 1 selected from the group consisting of:

GCTTTCCCGG AAACCTGCCG C,

CTCTGAGTAG CAGAGGAGCT C,

CCCCGACTCA CCTGGGAACA G,

GCTACACATG TCTATGGAGG G,

GGCAGAAATG TATGTGGGTG G,

ACACATACAC ACACACACAC A,

AGCCACCTGG GGGCCAAGGG G,

GGTTGCAACT CTGAG

CTATGGAGGG CCACTTC

GGGCCACTTC TTCTGTAA

CCCCCACCAC TTCCCCTCTC A

TCTGAGTAGC AGAGGAGCTC AG

GAGTAGCAGA GGAGC

GATGCTACAC ATGTC

GTTTAAGGCA GCATCCTAAG A,

TCACCCAAAG GTTTAGGCT A,

GCAATCATGA CTTCAAGAGT T,

GTTCACAACT GAAAAAGAAA G,

GCATGTCACA GCTGTAACAA A,

TGAAGTCAGC CAAGAACAGC T,

CGTTCTGCAC TTACCGTTTT G,

CAGCCAAGAA CAGCT

CAGCCAAGAA CAGCTGG

GATGTGAAGT CAGCCAA

CCCAAAGGTT TAGGCTTG

GAGTTCTTTT CACCC

GCCTGGGCGG GTATTCAGCT C,

AACCCTTATT TGTGTCCCAC C,

CCCAGGCATT TTAAGTTGCT G,

CACGAGGCCA CCACTCATCT C

CTTTGACTTC TTGCTCACAG C,

ACCTCCTCCA GTTCTCTCAT C,

TCCTGAAGCC AGTGAGGCCC G,

ACCTGTGTGT GCCTGGGAGG G,

CGTGAGGAGA AAATAGTGGT T,

CGACCATCTT CCGAGGCATT T,

CCACCACTCA TCTCG

CCCATTCACG AGGCCACC

CGAGGCCACC ACTC

CCTCCAGTTC TCTC

GCCAGTGAGG CCCG

CCTGGGAGGG TATTC

CCCTTATTTG TGTCCC

GGTTCCAAAA CCCTT

TTTGTGTCCC ACCTG

CATCTTCCCA GGCAT

wherein the sequences as listed are in the 5' to 3' direction.

9. The oligonucleotide of Claim 1 wherein the oligonucleotide contains at least one modification selected from the group consisting of modifications to the oligonucleotide phosphate backbone, including but not limited to oligonucleotides where at least one internucleoside

linkage is of the 2',5' composition, modifications to the termini, sugar moieties, individual nucleic acid bases, and combinations thereof.

10. The oligonucleotide of Claim 1 wherein the oligonucleotide is a phosphorothioate oligonucleotide or contains at least one phosphorothioate linkage.

11. A pharmaceutical composition comprising a pharmaceutical carrier and a therapeutically effective amount of at least one oligonucleotide having a nucleotide sequence at least substantially complementary to at least a portion of the mRNA transcript of the human ICAM-I, E-selectin, or VCAM-1 genes, said oligonucleotides being hybridizable to at least a portion of said mRNA transcripts.

12. The pharmaceutical composition of Claim 11 wherein the at least one oligonucleotide has a nucleotide sequence at least substantially complementary to a portion of human ICAM-I, E-selectin, or VCAM-1 mRNA transcripts including the translation initiation codons of said transcripts.

13. The pharmaceutical composition of Claim 11 wherein said composition is in the form of an acceptable solution for intravenous injection.

14. The pharmaceutical composition of Claim 11 wherein said composition is in the form of an acceptable solution, cream, or aerosol suitable for topical application.

15. The pharmaceutical composition of Claim 12 further comprising a cell membrane permeabilizing agent, wetting agents, or

drying agents.

16. The pharmaceutical composition of Claim 11 wherein the at least one oligonucleotide and carrier are encapsulated in a liposome.

17. The pharmaceutical composition of Claim 11 wherein the at least one oligonucleotide is conjugated to folate.

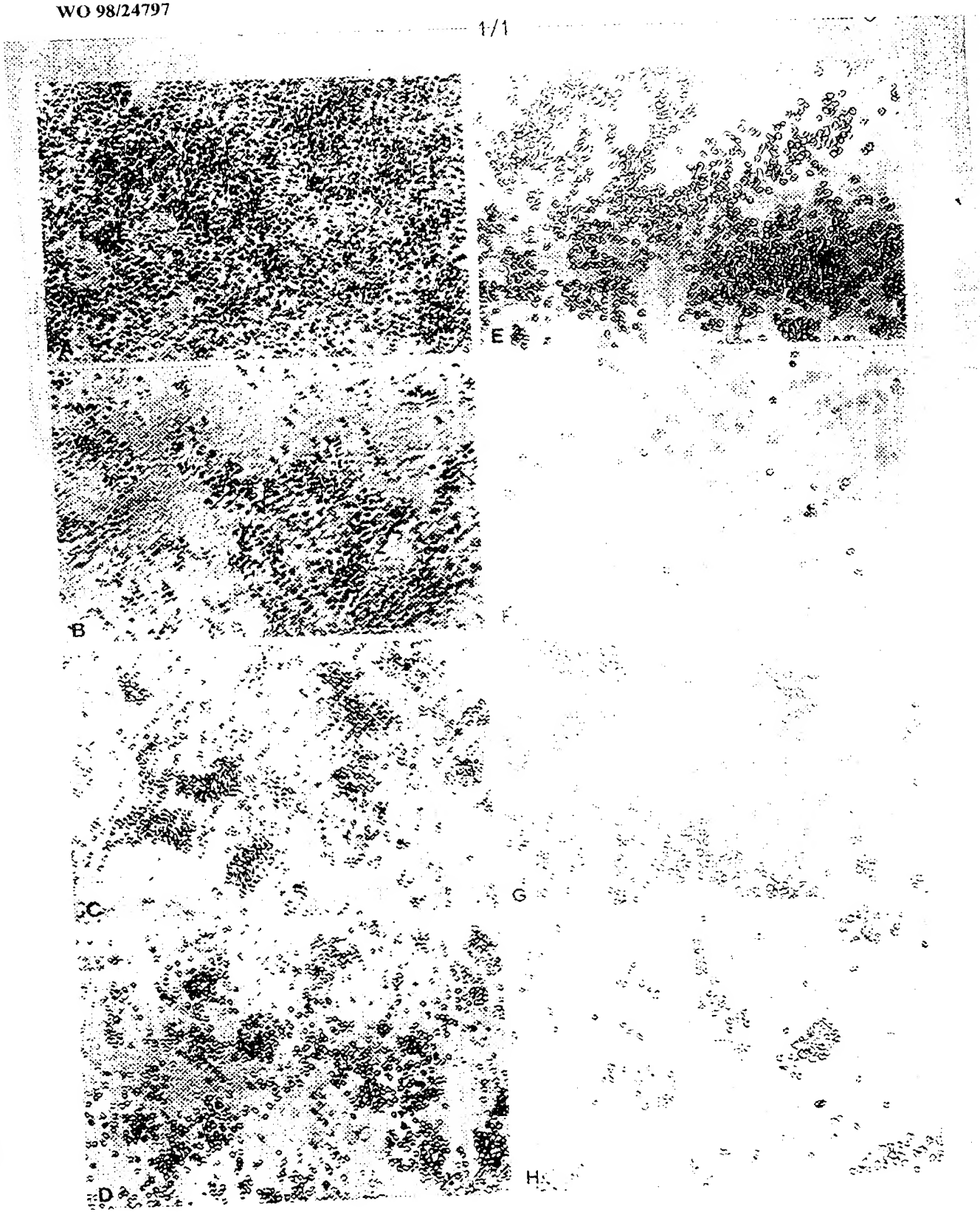
18. A method for treating a human patient in need of such treatment comprising administering to said patient a therapeutically effective amount of a composition comprising a pharmaceutical carrier and an oligonucleotide having a sequence at least substantially complementary to the human ICAM-I, E-selectin, or VCAM-1 mRNA transcripts, whereby said oligonucleotide penetrates the patient's cells and specifically hybridizes with substantially complementary mRNAs so as to block mRNA translation of the transcripts encoding ICAM-I, E-selectin, or VCAM-1, thereby inhibiting the synthesis of ICAM-I, E-selectin, or VCAM-1.

19. The method of Claim 18 wherein the composition is administered in the form of an i.v. solution.

20. The method of Claim 18 wherein the composition is administered by surgical implantation of a drug releasing agent at/or within the vasculature of the patient.

21. The method of Claim 18 wherein the composition is administered in the form of a topical application.

22. The method of Claim 18 wherein the composition is administered by encapsulation in a liposome.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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19194

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04; A61K 48/00
US CL : 536/23.1, 24.3, 24.31; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 24.3, 24.31; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MPSRCH
search term: antisense, oligonucleotide, ICAM-1, E-selectin, VCAM-1, ELAM-1, cell adhesion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, E --- Y, E	US 5,591,623 A (BENNETT et al.) 07 January 1997. See entire document.	1-7, 9-13, 18-19 --- 8, 14-17, 20-22
X -- Y	CHIANG et al. Antisense Oligonucleotides Inhibit Intercellular Adhesion Molecule 1 Expression by Two Distinct Mechanisms. The Journal of Biological Chemistry, 25 September 1991, Vol. 266, No. 27, pages 18162-18171. See entire document.	1-7, 9-13 --- 8, 14-22
Y	US 5,284,931 A (SPRINGER et al.) 08 February 1994. See entire document.	18-22

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 MARCH 1997	Date of mailing of the international search report 15 APR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MINH-TAM B. DAVIS Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/19194

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, E	US 5,593,974 A (ROSENBERG et al.) 14 January 1997. See entire document.	14-16
Y	LEAMON et al. Delivery of macromolecules into living cells: A method that exploits folate receptor endocytosis. Proceedings of the National Academy of Science USA, July 1991, Vol. 88, pages 5572-5576. See entire document.	17